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EPIDEMIOLOGY

Combined effects of *MDM2* SNP309 and *TP53* R72P polymorphisms, and soy isoflavones on breast cancer risk among Chinese women in Singapore

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Abstract The MDM2 oncoprotein regulates the p53 pathway and, while functional polymorphisms of the MDM2 and p53 genes have been investigated for association with breast cancer risk, results are largely null or non-conclusive. We have earlier reported that the increased intake of soy isoflavones reduces risk of postmenopausal breast cancer, and experimental studies suggest that dietary isoflavones can down-regulate the expression of the MDM2 oncoprotein. In this study, we investigated the association between the *MDM2* SNP309 and *TP53* R72P polymorphisms and breast cancer risk using a case–control study of 403 cases and 662 controls nested among 35,303 women in The Singapore Chinese Health Study, a population-based, prospective cohort of middle-aged and elderly men and women who have been continuously followed since 1993. The G allele of the *TP53* R72P polymorphism and T allele of the *MDM2* SNP309 polymorphism were putative high-risk alleles and exhibited a combined gene–dose-dependent joint effect on breast cancer risk that was more clearly observed in postmenopausal women. Among postmenopausal women, the simultaneous presence of G allele in *TP53* and T allele in *MDM2* polymorphisms was associated with an odds ratio (OR) of 2.42 [95% confidence interval (CI) 1.06–5.50]. Furthermore, the protective effect of dietary soy isoflavones on postmenopausal breast cancer was mainly confined to women homozygous for the high activity *MDM2* allele (GG genotype). In this genetic subgroup, women consuming levels of soy isoflavones above the median level exhibited risk that was half of those with below median intake (OR 0.52; 95% CI 0.28–0.99). Our findings support experimental data implicating combined effects of MDM2 protein and the p53-mediated pathway in breast carcinogenesis, and suggest that soy isoflavones may exert protective effect via down-regulation of the MDM2 protein.

Keywords MDM2 · p53 · Soy isoflavones · Breast cancer · Chinese

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Introduction

The activation of p53 protein upon cellular stress such as DNA damage and oncogene activation leads to induction of cell–cycle arrest and the activation of apoptotic cell death, and may account for the role of this tumor suppressor protein in preventing the accumulation of genomic alterations and tumor development [1]. Somatic inactivating mutations of the p53 gene are found in over 50% of all human tumors [2]. Hence, naturally occurring functional

polymorphisms of the p53 gene have also been investigated for possible association with human susceptibility to cancer. The p53 gene has a single base change of G to C at exon 4 codon 72, known as the *TP53* R72P polymorphism, which causes alteration of amino acid residue from arginine to proline [3]. Although both forms do not differ in their ability to bind to DNA in a sequence-specific manner, these two p53 variants differ in their abilities to bind components of the transcriptional machinery, to activate transcription, to induce apoptosis, and to repress the transformation of primary cells [4].

An important regulator of the p53 pathway is the MDM2 protein which is an E3 ubiquitin ligase that can inhibit p53 activity by promoting ubiquitination and degradation of p53 protein [5]. MDM2 binds to p53 and regulates its cellular localization, stability, and activity. Hence, the levels of the MDM2 protein in a cell or organism seem to have a large effect on the antitumorigenic activity of p53 [6]. In animal studies, overexpression of MDM2 leads to tumorigenesis in susceptible mice [7]; from human data, amplification of the *MDM2* gene has been documented in several tumor types [8–10]. The *MDM2* SNP309 polymorphism is a T to G change at nucleotide 309 in the first intron (rs2279744). Compared with the T allele, the G variant has been shown to have increased expression of MDM2, particularly in response to estrogens, and is associated with a decrease in the levels of p53 protein levels and an attenuation in p53-mediated transcription of genes [6, 11].

Despite abundant experimental data suggesting that the attenuation of the p53 stress response pathway by the MDM2 protein has significant effect on breast carcinogenesis, epidemiologic associations of the *MDM2* SNP309, and *TP53* R72P polymorphisms with breast cancer risk, either separately or in interaction, have been largely null or non-conclusive [12–14]. In a study that examined the interaction between these two genes in breast cancer risk among the US women in the Nurses Health Studies, none of the combined genotypes of these two gene polymorphisms reached statistical significance, and the direction of any possible interaction was inconsistent and uninterpretable [12].

In this study, we investigated the individual and combined effects of *MDM2* SNP309 and *TP53* R72P polymorphisms on breast cancer risk in a case–control study nested among female participants of the Singapore Chinese Health Study, a prospective cohort study of diet and cancer. Genistein, a dietary isoflavone, has been shown to downregulate the expression of the *MDM2* oncogene at both the transcriptional and translational level [15], and the inhibition of the MDM2 protein has in turn been associated with antitumor activities in a breast cancer model [16]. Earlier, we had reported that postmenopausal women with above median intake of soy had a statistically significant, 26% reduction in breast cancer risk compared with women with lower intake [17]. Thus, in this study, we also explored whether the beneficial effect of dietary soy on breast cancer is mediated through an MDM2/TP53 driven pathway.

Materials and methods

Study subjects

The study design and subject recruitment of the Singapore Chinese Health Study have been previously described [18]. In brief, 63,257 Chinese women and men, aged 45–74 years belonging to the Hokkien or Cantonese dialect group, were enrolled in the study between April 1993 and December 1998. At recruitment, information on lifestyle factors, usual diet, and reproductive history (for women only) was obtained through in-person interviews. The questionnaire also included semi-quantitative food frequency questionnaire (FFQ) assessing current intake patterns, which was subsequently validated against a series of 24-h-diet recalls among a subpopulation drawn randomly among the cohort participants [18]. Written informed consent was obtained from all the participants in the Singapore Chinese Health Study. This study was conducted under the ethical approval for the Singapore Chinese Health Study, and approved by the Institutional Review Boards at the National University of Singapore and the University of Minnesota.

Previously, we have measured concentrations of genistein, daidzein, and glycitein in the market samples of the seven common soyfoods in Singapore [19]. Total soy isoflavone intake for a given subject was computed based on the subject's response to the semi-quantitative food frequency questionnaire, and the summation of genistein, daidzein, and glycitein contents of the soyfoods in the Singapore Food Composition Database [18, 20]. Earlier, in a random sample of cohort subjects, we have shown the presence of a statistically significant association between urinary levels of isoflavones and dietary soy intake estimated from the food frequency questionnaire [21].

Between April 1994 and December 1999, we attempted to collect blood and single-void urine specimens from a random 3% sample of study enrollees. Details of the biospecimen collection, processing, and storage procedures have been described previously [22]. If the subject refused to donate blood, then buccal cell samples were requested and collected, if the subject consented. Out of 1,059 female cohort participants contacted for biospecimen donation, blood ($n = 514$) or buccal cells ($n = 164$) were collected from 678 subjects, representing a participation rate of 64%. The control group for the present study comprised this subcohort of women free of a history of breast cancer as of

Table 1 Distributions of known risk factors for breast cancer among cases and controls

	All women			Postmenopausal women at recruitment		
	Case	Control	OR (95% CI)[a]	Case	Control	OR (95% CI)[a]
Total subjects	403	662		281	462	
Age (years) at menarche						
<13	67	98	1.00	41	53	1.00
13–14	173	260	1.01 (0.70–1.46)	109	172	0.84 (0.52–1.35)
15–16	124	207	0.95 (0.64–1.42)	98	160	0.88 (0.54–1.45)
17+	39	97	0.65 (0.39–1.07)	33	77	0.62 (0.34–1.12)
P for trend			0.114			0.202
Number of live births						
None	49	48	1.00	36	30	1.00
1–2	143	183	0.81 (0.51–1.28)	78	95	0.71 (0.40–1.26)
3–4	136	262	0.54 (0.34–0.86)	96	180	0.48 (0.28–0.83)
5+	75	169	0.44 (0.26–0.73)	71	157	0.41 (0.23–0.74)
P for trend			0.0001			0.0007
Age (years) at first live birth						
≤20	56	123	1.00	46	99	1.00
21–25	129	247	1.12 (0.76–1.65)	89	182	1.02 (0.66–1.58)
26–30	111	183	1.27 (0.84–1.92)	75	117	1.27 (0.79–2.05)
31+	57	60	1.95 (1.18–3.21)	34	34	1.96 (1.06–3.61)
Nulliparous	49	48	2.03 (1.19–3.45)	36	30	2.29 (1.23–4.28)
P for trend			0.0008			0.001
Family history of breast cancer						
No	395	654	1.00	274	457	1.00
Yes	8	8	1.49 (0.55–4.03)	7	5	2.02 (0.63–6.48)
BMI (kg/cm^2)						
<20	47	94	1.00	27	54	1.00
20– < 24	227	371	1.26 (0.85–1.86)	160	269	1.20 (0.73–2.00)
24– < 28	92	153	1.27 (0.82–1.96)	67	104	1.32 (0.75–2.30)
28+	37	44	1.82 (1.04–3.21)	27	35	1.63 (0.82–3.24)
P for trend			0.074			0.152
Age (years) at menopause (postmenopausal women only)						
<50				102	200	1.00
50–54				148	233	1.28 (0.93–1.77)
55+				31	29	2.27 (1.29–4.01)
P for trend						0.006

[a] Adjusted for age at recruitment, dialect group and level of education, *OR* odds ratio, *CI* confidence interval

December 31, 2007. There were 662 subjects who satisfied these criteria.

We identified incident breast cancer cases occurring within the Singapore Chinese Health Study cohort through the population-based cancer registry in Singapore. The nationwide cancer registry has been in place since 1968 and has been shown to be comprehensive in its recording of cancer cases [23]. As of December 31, 2007, there were 783 incidences of female breast cancer cases in this cohort; among them, 403 cases (51.5%) had also given us blood or buccal specimens for genotyping. Histological and staging information of these 403 breast cancer diagnoses were confirmed by manual review of the pathology reports and clinical charts. Compared with breast cancer patients who donated a blood or buccal sample, those who did not were less educated (37 versus 31% had no formal education). More Cantonese gave biospecimens (55.7%) compared with Hokkiens (44.3%). Those who donated biospecimens also were a few years younger at diagnosis compared to those who did not donate (mean, 61 versus 63 years).

Genotyping methods

The *MDM2* SNP309 (rs2279744) and *TP53* R72P (rs1042522) polymorphisms were genotyped using the fluorogenic 5′-nuclease assay (TaqMan Assay) [24]. The TaqMan assays were performed using a TaqMan 2X Universal PCR Master Mix (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. The oligonucleotide primers for amplification of the polymorphic region of *MDM2* were GC148for (5′-TTCAGGGTAAAG GTCACGGG-3′) and GC148rev (5′-ACTACGCGCAGCG TTCACAC-3′) and for *TP53* were GC155for (5′-TCCCC GGACGATATTGAACAA-3′) and GC155rev (5′-GGCCG CCGGTGTAGGA-3′). In addition, the fluorogenic oligonucleotide MGB probes used to detect each of the alleles for *MDM2* were GC148F (5′-CGCCGCAGCGGCC-3′) labeled with 6-FAM to detect the G allele and GC148 V (5′- CGCCGAAGCGGCC -3′) labeled with VIC to detect the *T* allele (Applied Biosystems). The MGB probes for *TP53* were GC155F (5′-TGCTCCCCGCGTGGC-3′) labeled with 6-FAM to detect the Arg (R) allele and GC155 V (5′-TGCTCCCCCCGTGGC-3′) labeled with VIC to detect the Pro (P) allele. PCR amplification using ~10 ng of genomic DNA was performed in a thermal cycler with an initial step of 95°C for 10 min followed by 50 cycles of 95°C for 25 s and Tm °C for 1 min (Tm = 66 for *MDM2* and 60 for *TP53*). The fluorescence profile of each well was measured in an ABI 7900HT Sequence Detection System and the results analyzed with Sequence Detection Software (Applied Biosystems). Experimental samples were compared with 12 controls to identify the three genotypes at each locus. Any of the samples that were outside the parameters defined by the controls were identified as non-informative and were re-tested.

A total of 54 (5.1%) subjects had non-informative genotype for *MDM2* SNP309 polymorphism, 38 (3.6%) had non-informative genotype for *TP53* R72P polymorphisms and 12 subjects (1.1%) had non-informative genotypes for both genes. These subjects were excluded from the statistical analysis accordingly.

Statistical analysis

Data were analyzed by standard methods for unmatched case–control studies [25]. Unconditional logistic regression models were employed to examine the associations between *MDM2* and *TP53* genotypes, separately and in combination, and breast cancer risk. The associations were measured by odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) and *P* values. Age at recruitment, dialect group (Cantonese, Hokkien), level of education (none, primary, secondary school or higher), age at first livebirth (20 years or younger, 21–25 years, 26–30 years, 31 years or older, nulliparous), soy isoflavone intake (quartiles), and body mass index (<20 kg/m^2, 20+ kg/m^2) were covariates in all logistic regression models. The analyses involving women who were postmenopausal at recruitment also included age at menopause (<50 years, 50–54 years, and 55 years or older) as a covariate. The same methodology was used to examine if the soy-breast cancer association differed by *MDM2* or *TP53* genotypes [25]. All covariates described above were included in these logistic regression runs.

Statistical analysis was carried out using the SAS software Version 9.1 (SAS Institute, Cary, NC). All the reported *P* values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Among the subjects, 69.8% were postmenopausal at recruitment. The mean ages at biospecimen collection for cases and controls were 61.3 [standard deviation (SD) 8.0] and 58.8 (SD 8.3) years, respectively. Among all the women with breast cancer, the mean age at diagnosis was 61.5 years (SD 8.2 years). Among the women with breast cancer who were already postmenopausal at recruitment, the mean age at diagnosis was 64.7 years (standard deviation 7.4 years). Similar to our earlier results [22, 26], increasing age at menarche and increasing number of live births were associated with reduction in breast cancer risk. Likewise, nulliparity, late age at first live birth, family history of breast cancer, late age at menopause, and increased body mass index were all associated with increased breast cancer risk (Table 1).

Among the control subjects, the frequencies of the C and G alleles of the *TP53* R72P polymorphism were 0.47 and 0.53, while the frequencies of the G and T alleles of the *MDM2* SNP309 polymorphism were 0.53 and 0.47, respectively. All genotypic distributions were in Hardy–Weinberg equilibrium (*P* value = 0.9 for *TP53* R72P and *P* = 0.6 for *MDM* SNP309). For the *TP53* R72P polymorphism, women possessing at least one copy of the G allele (putative high-risk genotype) had an increase in breast cancer risk (OR 1.19; 95% CI 0.86–1.64) relative to the CC genotype. For the *MDM2* SNP309 polymorphism, women with at least one copy of the T allele (putative high-risk genotype) showed a higher increase in breast cancer risk compared with those homozygous for the G allele (OR 1.20; 95% CI 0.90–1.62) (Table 2).

The relative risk for *TP53* low-risk genotype jointly with *MDM2* high-risk genotype is similar to that for *TP53* high-risk genotype jointly with *MDM2* low-risk genotype (Table 2). When these two genotypes were grouped (both

Table 2 *TP53* R72P and *MDM2* SNP309 genotypes in relation to breast cancer risk

	All women			Postmenopausal women		
	Cases	Controls	OR(95% CI)[a]	Cases	Controls	OR(95% CI)[b]
TP53 R72P genotype						
CC (0)	73	145	1.00	47	105	1.00
GC	197	319	1.21 (0.86–1.69)	141	212	1.42 (0.94–2.15)
GG	102	179	1.15 (0.79–1.68)	71	130	1.19 (0.75–1.90)
GC or GG (1)	299	498	1.19 (0.86–1.64)	212	342	1.34 (0.90–1.98)
MDM2 SNP309 genotype						
GG (0)	96	174	1.00	65	120	1.00
GT	212	300	1.28 (0.94–1.75)	150	212	1.31 (0.90–1.91)
TT	77	140	1.03 (0.71–1.51)	53	95	1.03 (0.65–1.64)
TT or GT (1)	289	440	1.20 (0.90–1.62)	203	307	1.22 (0.85–1.75)
TP53/MDM2 genotypes						
0/0 (0 high-risk genotype)	17	40	1.00	8	29	1.00
1/0 or 0/1 (1 high-risk genotype)	123	226	1.30 (0.70–2.40)	88	156	1.99 (0.86–4.62)
1/0	70	130	1.28 (0.67–2.43)	51	87	2.05 (0.86–4.88)
0/1	53	96	1.33 (0.68–2.59)	37	69	1.92 (0.78–4.73)
1/1 (2 high-risk genotypes)	221	334	1.60 (0.88–2.92)	156	230	2.42 (1.06–5.50)
P for trend[c]			0.051			0.036

[a] Adjusted for age at recruitment, dialect group, level of education, body mass index, age at first livebirth and soy isoflavone intake, *OR* odds ratio, *CI* confidence interval

[b] Additionally adjusted for age at menopause, *OR* odds ratio, *CI* confidence interval

[c] For association between number of high-risk genotypes (0, 1 or 2) and breast cancer risk

are characterized by the presence of one high-risk genotype), the OR (95% CI) for breast cancer risk was 1.30 (0.70–2.40). The highest risk for breast cancer was observed in women possessing both *TP53* and *MDM2* high-risk genotypes (OR 1.60; 95% CI 0.88–2.92) (*P* for trend = 0.051). The association became stronger when the analysis was confined to postmenopausal women; those possessing both high-risk genotypes experienced a statistically significant, 2.42 times increased risk for breast cancer relative to women devoid of high-risk genotypes (95% CI 1.06–5.50). The association between the number of high risk genotypes and postmenopausal breast cancer risk was statistically significant (*P* for trend = 0.036) (Table 2).

Earlier, based on the entire cohort of women [17], we reported that postmenopausal women with above- versus below median intake of soy isoflavones experienced a statistically significant, reduced risk of breast cancer (relative risk: 0.74; 95% CI 0.61–0.90). In this nested case–control study within the cohort, we noted remarkably similar results (OR 0.74; 95% CI 0.54–1.01) (Table 3). We examined whether *MDM2* genotype exerted an influence on the dietary soy isoflavone-postmenopausal breast cancer risk association. We noted that the strong protective effect of dietary soy isoflavones on postmenopausal breast cancer was mainly confined to women possessing the GG genotype that was associated with increased MDM2 protein expression (Table 3). There was no evidence that *TP53* genotype exerted any influence on the dietary soy isoflavone-postmenopausal breast cancer association (Table 3).

Discussion

The present study demonstrated that *MDM2* SNP309 and *TP53* R72P polymorphisms jointly exhibited a gene–dose-dependent association with breast cancer, in particular postmenopausal breast cancer. The highest risk was observed in women possessing both the G allele (functionality still controversial for breast cancer) of the *TP53* gene and the low-activity T allele of the *MDM2* gene. Furthermore, we showed that the protective effect of dietary soy isoflavones on postmenopausal breast cancer was mainly confined to women possessing the high-activity GG genotype of the *MDM2* gene.

The three major outcomes of the p53 stress response are cell–cycle arrest, cellular senescence, and apoptosis. The arginine variant coded by the G allele is a stronger and faster inducer of apoptosis, and more efficient in suppressing oncogene-induced transformation than the proline variant coded by the *C* allele [4, 27]. In contrast, the proline

Table 3 Effects of soy intake in relation with *TP53* R72P and *MDM2* SNP309 genotypes on breast cancer risk among postmenopausal women

	Soy isoflavones	Cases	Controls	OR(95% CI)[a]
All postmenopausal women	<10.6 mg/1000 kcal per day	161	242	1.00
	>=10.6 mg/1000 kcal per day	120	220	0.74 (0.54–1.01)
MDM2 SNP309 GG	<10.6 mg/1000 kcal per day	36	49	1.00
	>=10.6 mg/1000 kcal per day	29	71	0.52 (0.28–0.99)
MDM2 SNP309 GT or TT	<10.6 mg/1000 kcal per day	119	175	1.00
	>=10.6 mg/1000 kcal per day	84	132	0.84 (0.58–1.22)
TP53 R72P CC	<10.6 mg/1000 kcal per day	28	53	1.00
	>=10.6 mg/1000 kcal per day	19	52	0.69 (0.33–1.47)
TP53 R72P GC or GG	<10.6 mg/1000 kcal per day	120	183	1.00
	>=10.6 mg/1000 kcal per day	92	159	0.79 (0.56–1.13)

[a] Adjusted for age at recruitment, dialect group, level of education, body mass index, age at first livebirth and age at menopause, *OR* odds ratio, *CI* confidence interval

variant is a stronger inducer of transcription and more efficient in inducing cell-cycle arrest [4, 27]. Owing to the difference between the two p53 polymorphic variants in their biochemical properties and biological effects on cell cycle progression, it is difficult to define the functionally-impaired p53 protein as this may differ according to which biological deficiency in the p53-driven mechanistic pathway is dominant in the carcinogenesis of different subtypes of breast cancer. This may also be the reason for the lack of consistent replication in the reported gene-cancer associations in the literature, although the *TP53* R72P polymorphism has been tested extensively for several cancers, such as lung, cervical, colorectal, and bladder cancers [28–33]. Furthermore, studies that showed positive association between p53 polymorphism and cancer risk were also contradictory with regard to the identity of the high-risk allele, with some studies showing the G allele to increase cancer risk [31, 34], while others showed the C allele to be the high-risk allele [35–37]. In the most recent meta-analysis of 21 studies involving 24,063 subjects, the biggest to date, there was no association between *TP53* R72P polymorphism and breast cancer risk [38], which concurs with an earlier meta-analysis of 17 case–control studies that also concluded an overall null association [39]. In our study, the increased risk of breast cancer among women with the G allele of the *TP53* R72P polymorphism, albeit not reaching statistical significance, suggests that the p53 Arg variant protein, which is less efficient at inducing a growth arrest than the p53 Pro variant protein, increases breast cancer susceptibility. Consistent with our findings, another study among Singapore Chinese showed that the silent G allele that codes for the Arg variant in healthy Chinese heterozygotes seemed to have been reactivated during breast cancer formation, suggesting a positive correlation between the G allele expression and breast cancer risk [40].

For the *MDM2* SNP309 polymorphism, the G allele increases the affinity of a well-described cotranscriptional activator of nuclear hormone receptors (Sp1) and results in increased expression of the MDM2 protein. Since the polymorphism sits in a promoter region regulated by hormonal signaling pathways, the *MDM2* SNP309 locus could also possibly affect how hormones, such as estrogen, affect tumorigenesis in humans [6, 11]. However, published case–control studies have yielded contradictory results, leading to essentially null associations in subsequent meta-analysis [41, 42]. Interestingly, a recent meta-analysis of 16 case–control studies showed that while there was essentially null association between the *MDM2* gene polymorphism and breast cancer risk in non-Chinese populations, there was a suggestion of increased risk with the G allele among Chinese populations [42].

In the combined effects of *MDM2* SNP309 and *TP53* R72P polymorphisms on breast cancer risk, if the p53 Arg variant protein is indeed implicated in breast carcinogenesis, it is reasonable to postulate that the genetic combination with increased breast cancer risk would be the G allele for *TP53* polymorphism that codes for the p53 Arg variant protein, and the T allele for *MDM2* polymorphism that is associated with lower expression of the MDM2 protein and an attenuation in the degradation of the p53 protein. Conversely, our study results suggest that higher expression of the MDM2 protein and increased degradation of the p53 Pro variant protein in women homozygous for the *MDM2* SNP309 G allele as well as for the *TP53* R72P *C* allele could confer protective effect for breast cancer. In a recent pooled series from the Breast Cancer Association Consortium involving 5,191 cases and 3,834 controls, although none of the associations reached statistical significance, the genetic combination with the lowest risk was the GG genotype of the *MDM2* gene and the CC genotype of the *TP53* gene, entirely consistent with our observations [14]. Another study that examined survival of breast cancer patients showed that among patients homozygous for the *MDM2* T allele, mutant p53 status and aberrant p53 protein expression in breast tumors were associated with poor

survival [43]. Our results and some of the other studies cited suggest that the T allele-encoded MDM2 protein, in the presence of an impaired p53 pathway, may lead to increased stability and nuclear protein accumulation of the impaired p53 variant protein, and thus increase cancer susceptibility.

Epidemiologic studies among Asian populations that consume moderate to high amount of soy, including this cohort of Chinese women in Singapore, have shown that dietary soy may protect against postmenopausal breast cancer [17, 44]. While it remains possible that soy intake may be a marker of some other dietary or lifestyle factors that are causally related to breast cancer risk, experimental studies have provided strong evidence on plausible causal pathways through which dietary soy exerts its anti-carcinogenesis effects. Experimental studies have shown that soy isoflavones predominately bind to and activate ERβ [45, 46], which in turn has been associated with the upregulation of genes that leads to inhibition of proliferation in breast cancer cells [47–49]. Lately, experimental studies have revealed that the anti-cancer effects of dietary isoflavones may also be via ER-independent mechanisms, such as through the inhibition of tyrosine protein kinase, an enzyme frequently implicated in carcinogenesis [50], and also by their pro-apoptotic and antiangiogenic effects [51]. More recently, genistein, a dietary isoflavone, has been shown to down-regulate the expression of the *MDM2* oncogene at both the transcriptional and translational levels [15], and the inhibition of the expression of the MDM2 protein has in turn been associated with antitumor activities in a breast cancer model [16].

Our results showed a stronger soy-breast cancer association among women possessing the *MDM2* high-activity genotype (GG) relative to their counterparts with the low-activity genotypes (GC and CC). In other words, women with higher expression of the MDM2 protein may show a more prominent effect of soy isoflavone protection relative to women with lower expression. Our data lend support to the hypothesis that the protective effect of dietary soy on breast cancer development, at least partially, is mediated through the down-regulatory effect of soy isoflavones on MDM2 expression. There is no experimental evidence to link p53 to the biological effect of soy on breast cancer. As expected, we noted no difference in the soy-breast cancer association by *TP53* genotype. It would be of extreme interest to ascertain whether the cancer risk reduction associated with higher intake of soy isoflavone is restricted to wild-type p53 tumors in which down-regulation of MDM2 expression would be expected to have a beneficial effect.

The current study has several strengths. In this case–control study nested within a well-established cohort, soy consumption and other known environmental risk factors for breast cancer were assessed before cancer diagnosis, and hence can be presumed to be free of recall bias. The limitation of the study is its relatively small sample size of breast cancer cases. Hence, we interpret our observations with caution, viewing them principally as a hypothesis-generating set of findings that require confirmation by an independent group. Nonetheless, it is important to note that our novel findings possess biological plausibility, and are consistent with predictions based on recognized models of MDM2/p53-mediated pathways in breast carcinogenesis. Our observations on the MDM2 genotype's influence on the protective effect of dietary soy on breast cancer carries important scientific as well as public health implications.

In summary, the present study shows a combined effect of *MDM2* SNP309 and *TP53* R72P polymorphisms on breast cancer risk, and provides epidemiological validation showing that the biological interaction between MDM2 protein and the p53-mediated pathway may play a role in breast carcinogenesis. This is also the first epidemiologic report suggesting that the ability of soy isoflavones to down-regulate the MDM2 protein may be one etiologic mechanism through which the intake of soy is associated with breast cancer protection.

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Conflicts of interest The authors declare that they have no conflicts of interest.

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